Protocol-at-a-Glance

Sprint™ Titanium® 96 Plate Protocol-at-a-Glance

(PT3920-2)

The Protocol-at-a-Glance for the Sprint Titanium 96 Plate (Cat. No. 639567) is provided for your convenience.

Preparation of the Primer/Template Master Mix

The final reaction volume (containing primers, template, and PCR-grade water for a single reaction) should be 25 µl. Assemble a master mix containing the appropriate volume of water, primers, and/or template for the required number of PCR reactions. To ensure a sufficient volume of master mix, we recommend that you set up the master mix to provide the required volume for all your reactions, plus 10% extra.

Reconstitution of the Wells

1. Reconstitute each well of the Sprint Titanium 96 Plate with 25 µl of the master mix.
2. Pipet up and down several times or vortex the plate gently to reconstitute, and then seal the wells.
3. Briefly centrifuge the plates at 2,000 rpm for 5 min in a benchtop plate centrifuge to collect liquid at the bottom of the wells.
4. Begin thermal cycling using the guidelines provided below.

Recommended Cycling Parameters

Use the following guidelines when setting up your initial experiments with the Sprint Titanium 96 Plate. These are general guidelines—the optimal parameters may vary with different thermal cyclers and will depend on your particular primers, template, and other experimental variables.

Target Size	Cycle Parameters
<1 kb:	• 95°C for 1 min • 25–35 cycles[a] 95°C for 30 sec[b] 68°C for 1 min[c] • 68°C for 1 min[d]
1–5 kb:	• 95°C for 1 min • 25–30 cycles[a] 95°C for 30 sec[b] 68°C for 3 min[c] • 68°C for 3 min[d]

[a] 25 cycles for multiple-copy genes or medium-to-high abundance cDNAs; 30–35 cycles for single- or low-copy number genes or rare cDNAs. For most applications, we prefer two-step cycles (denaturation at T_1 followed by annealing and extension at T_2) instead of three-step cycles (denaturation at T_1 followed by annealing at T_2 followed by extension at T_3). Three-step cycles will be necessary when the T_m of the primers is less than 60–65°C and in certain special protocols.

[b] Use the minimal possible denaturation time. In some cases, better results may be obtained by modifying the denaturation step (94°C for 15 sec). Exposure of DNA to high temperatures causes some depurination of single-stranded DNA during denaturation, which eventually leads to strand scission. High temperature also leads to gradual loss of enzyme activity. Minimizing denaturation time is particularly important in experiments with very large templates where total cycling time can exceed 12 hr.

[c] Use the maximum possible annealing/extension temperature. See "Note a." Some researchers prefer to use an annealing/extension time equal to the expected target size (in kb) plus two minutes. We recommend using 1 min per kb of expected target.

[d] Optional: This final extension may reduce background in some cases.

Recommendations for Electrophoresis

For 0.3–1.5 kb inserts, we recommend the use of 1.5% agarose and ϕX174/Hae III size markers. For 0.5–5 kb inserts, we commend the use of 1.2% agarose and a 1 kb DNA ladder.

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Clontech PCR Systems

Product	Size	Cat. No.
Sprint Titanium *Taq* 384 Plate	384 rxns	639552
Titanium *Taq* DNA Polymerase	100 rxns	639208
	500 rxns	639209
	1,000 rxns	639242
Titanium *Taq* PCR Kit	30 rxns	639211
	100 rxns	639210
Sprint Advantage® 96 Plate	96 rxns	639550
Sprint Advantage Single Shots	8 rxns	639553
Advantage 2 Polymerase Mix	100 rxns	639201
	500 rxns	639202
Advantage 2 PCR Kit	30 rxns	639207
	100 rxns	639206
Advantage HF 2 PCR Kit	10 rxns	639124
	100 rxns	639123
Advantage GC 2 Polymerase Mix	100 rxns	639114
Advantage GC 2 PCR Kit	10 rxns	639120
	100 rxns	639119
Advantage Genomic Polymerase Mix	100 rxns	639110
Advantage Genomic PCR Kit	30 rxns	639104
	100 rxns	639103

This document has been reviewed and approved by the Clontech Quality Assurance Department